```
     FILE 'BIOSIS, MEDLINE, EMBASE, EMBAL, SCISEARCH, BIOTECHDS, CAPLUS'
     ENTERED AT 15:22:37 ON 06 OCT 2003
L1          5033 S BISULFITE? AND METHYL?
L2            23 S L1 AND (CY3? AND CY5)
L3            17 DUP REM L2 (6 DUPLICATES REMOVED)
L4            16 S L3 AND (PCR? OR AMPLIF?)
L5             1 S L1 AND (FLOURESC?)
L6           153 S L1 AND (PURIFICATION? OR SEPARATION?)
L7           151 S L6 NOT L3
L8            21 S L7 AND (OLIGONUCLEOTIDE? OR SOLID?)
L9            20 DUP REM L8 (1 DUPLICATE REMOVED)
L10           20 S L9 NOT L2
```

L Number	Hits	Search Text	DB	Time stamp
1	187	bisulfite and (methylat$4 SAME cytosine)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:31
2	165	(bisulfite and (methylat$4 SAME cytosine)) and (PCR or amplif$8)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:31
3	103	((bisulfite and (methylat$4 SAME cytosine)) and (PCR or amplif$8)) and (fluorescent)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:31
4	6	((bisulfite and (methylat$4 SAME cytosine)) and (PCR or amplif$8)) and (flourescent$3)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:31
5	109	((bisulfite and (methylat$4 SAME cytosine)) and (PCR or amplif$8)) and fluorescent$3	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:32
6	20	(((bisulfite and (methylat$4 SAME cytosine)) and (PCR or amplif$8)) and fluorescent$3) and (cy3 and cy5)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:56
7	2	wo near "9928498"	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:57

FILE 'BIOSIS, MEDLINE, EMBASE, EMBAL, SCISEARCH, BIOTECHDS, CAPLUS'
ENTERED AT 11:53:13 ON 06 OCT 2003
L1 7168 S **METHYLATION** (S) **CYTOSINE**?
L2 17 S L1 AND (**BISULFATE**?)
L3 16 DUP REM L2 (1 DUPLICATE REMOVED)
L4 8 S L3 AND (**PCR**? OR AMPLF?)
L5 0 S L4 AND **CY3**?
L6 8 S L3 NOT L4
L7 72 S SEPARATION AND (**METHYLAT**? (S) **CYTOSINE**?)
L8 28 S L7 AND (**ELECTROPHORESIS** OR **HPLC**? OR **CPE**? OR (CAPLLIARY ADJ1
L9 0 S L8 AND (**CY3** OR **CY5**)
L10 0 S L9 AND (**DCTP**? OR **DGTP**?)
L11 0 S L8 AND (**DCTP**? OR **DGTP**?)
L12 18 DUP REM L8 (10 DUPLICATES REMOVED)
L13 916 S **CY3** AND **CY5**
L14 21 S L13 AND (METHYL? AND **CYTOSINE**?)
L15 8 S L4 AND **BISULFATE**?
L16 8 DUP REM L15 (0 DUPLICATES REMOVED)
L17 0 S L14 AND **BISULFATE**?
L18 19 DUP REM L14 (2 DUPLICATES REMOVED)

L4 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:87207 BIOSIS
DOCUMENT NUMBER: PREV199799378920
TITLE: Rapid analysis of DNA **methylation** using new restriction enzyme sites created by bisulfite modification.
AUTHOR(S): Sadri, Ramin; Hornsby, Peter J. (1)
CORPORATE SOURCE: (1) Dep. Cell Biol., Baylor Coll. Med., 1 Baylor Plaza M320, Houston, TX 77030 USA
SOURCE: Nucleic Acids Research, (1996) Vol. 24, No. 24, pp. 5058-5059.
ISSN: 0305-1048.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Bisulfite converts non-**methylated** **cytosine** in DNA to uracil leaving 5-methyl**cytosine** unaltered. Here, predicted changes in restriction enzyme sites following reaction of genomic DNA with bisulfite and **amplification** of the product by the polymerase chain reaction (**PCR**) were used to assess the **methylation** of CpG sites. This procedure differs from conventional DNA **methylation** analysis by **methylation**-sensitive restriction enzymes because it does not rely on an absence of cleavage to detect **methylated** sites, the two strands of. . . different restriction enzyme sites and may be differentially

analyzed, and closely related sequences may be separately analyzed by using specific **PCR** primers.

L4 ANSWER 6 OF 8 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1997-02964 BIOTECHDS
TITLE: **Methylation** analysis using **bisulfate** genomic sequencing: application to small numbers of intact cells; mouse Xist gene **methylation** profile by polymerase chain reaction
AUTHOR: McDonald L E; *Kay G F
CORPORATE SOURCE: Queensland-Inst.Med.Res.; Roy.Brisbane-Hosp.
LOCATION: Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Queensland, 4029, Australia.
Email: grahamk@qimr.edu.au
SOURCE: BioTechniques; (1997) 22, 2, 272,273
CODEN: BTNQDO
ISSN: 0736-6205
DOCUMENT TYPE: Journal
LANGUAGE: English
TI **Methylation** analysis using **bisulfate** genomic sequencing: application to small numbers of intact cells; mouse Xist gene **methylation** profile by polymerase chain reaction
AB **Bisulfate** genomic sequencing was applied to samples containing 50-200 intact cells to determine the **methylation** status of each **cytosine** residue in a defined sequence for individual strands of genomic DNA. Bisulfite-modified DNA is subjected to polymerase chain reaction (**PCR**) to yield products in which uracil residues are **amplified** as thymine and 5-methyl**cytosine** residues only are **amplified** as **cytosine**. This method allows the investigation of **methylation** in the control of gene expression in germ cells, embryogenesis, inherited diseases and cancers, when samples are limiting. This method was used to generate **methylation** profiles for a mouse Xist gene (569 bp DNA fragment) in mouse germ cells and during implantation development in order to study the role of **methylation** in the genomic imprinting of this gene. Genomic DNA was prepared from cells treated with a lysis buffer containing protease-K and guanidine hydrochloride. Isolated genomic DNA was modified with **bisulfate**, denatured by restriction endonuclease digestion and subjected to touchdown **PCR**. This method resulted in complete conversion of **cytosine** residues for all samples analyzed. (12 ref)

L6 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:740564 CAPLUS
DOCUMENT NUMBER: 132:246749

TITLE: Analysis of in vivo **methylation**
AUTHOR(S): Dahl, Hans-Henrik M.; Hutchison, Wendy M.
CORPORATE SOURCE: The Murdoch Institute, Royal Children's Hospital, Melbourne, Australia
SOURCE: Methods in Molecular Biology (Totowa, New Jersey) (2000), 130(Transcription Factor Protocols), 47-57
CODEN: MMBIED; ISSN: 1064-3745
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT
AB A review with 19 refs. A no. of methods are available to detect **methylated** **cytosines** (as 5-methyl**cytosine**, 5-MeC) in DNA and most are based on the use of **methylation**-sensitive restriction enzymes or genomic sequencing protocols. Recently, a simple and efficient genomic sequencing technol. has been developed. It is based in the observation that sodium **bisulfate**, followed by alk. treatment, converts **cytosine** residues in single stranded DNA to uracil under condition where 5-MeC is unreactive. Modifications of the bisulfite genomic sequencing technique are outlined.
IT 7681-38-1, Sodium **bisulfate**
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(anal. of in vivo **methylation** by bisulfite genomic sequencing)

L6 ANSWER 6 OF 8 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1999-03515 BIOTECHDS
TITLE: Detecting differences in **methylation** of DNA at specific CpG sites;
by polymerase chain reaction, used for cancer diagnosis
AUTHOR: Gonzalgo M L; Jones P A; Liang G
PATENT ASSIGNEE: Univ.Southern-California
LOCATION: Los Angeles, CA, USA.
PATENT INFO: WO 9856952 17 Dec 1998
APPLICATION INFO: WO 1998-US11896 9 Jun 1998
PRIORITY INFO: US 1997-49231 9 Jun 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1999-080918 [07]
AB A method for determining DNA **methylation** patterns at **cytosine** sites is claimed. It involves reacting DNA from a sample to be assayed, with sodium **bisulfate**, to convert un**methylated** **cytosine** to uracil, leaving 5-methyl**cytosine** unconverted. DNA primers specific for the converted sample are then used

to perform a polymerase chain. . . be measured by incorporating various 32P labeled dNTPs. Also claimed is a Ms-SNuPE primer sequence that hybridizes with a specific **cytosine** codon in a target CpG site, and that corresponds to a frequently hyper**methylat**ed gene sequence just upstream of the target. . . site by locating a hyper**methylat**ed CpG island in a somatic gene derived from malignant tissue. This is used to detect **methylation** changes associated with oncogenic transformation, and therefore to detect cancer. (19pp)

L12 ANSWER 8 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5
ACCESSION NUMBER: 2000:478524 BIOSIS
DOCUMENT NUMBER: PREV200000478524
TITLE: Rapid quantification of DNA **methylation** by high performance **capillary electrophoresis**.
AUTHOR(S): Fraga, Mario F.; Rodriguez, Roberto; Canal, Maria Jesus (1)
CORPORATE SOURCE: (1) Dpto. B.O.S. (Lab. Fisiologia Vegetal), Universidad de Oviedo, C/Catedratico Rodrigo Uria s/n, E-33071, Oviedo Spain
SOURCE: **Electrophoresis**, (August, 2000) Vol. 21, No. 14, pp. 2990-2994. print.
ISSN: 0173-0835.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
TI Rapid quantification of DNA **methylation** by high performance **capillary electrophoresis**.
AB The actual methods to evaluate total DNA **methylation** based on high performance liquid chromatography (**HPLC**) are long and tedious due to the specific running buffers required. In this work, a new open-tube **capillary electrophoresis** system has been applied to the separation of acid hydrolyzed genomic DNA and so, to the evaluation of genomic DNA **methylation**. Several running conditions were tested but separation of **cytosine** and 5-methyl-**cytosine** was only possible by sodium dodecyl sulfate (SDS) micelle system. The importance of sample dissolution preparation has also been demonstrated. The results of this study open up the possibility of quantification of the relative **methylation** degree of rapid genomic DNA by a simple method based on high performance **capillary electrophoresis** (HPCE).

L12 ANSWER 9 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:197982 BIOSIS

DOCUMENT NUMBER: PREV200000197982
TITLE: C5 **cytosine methylation** at CpG sites enhances sequence selectivity of mitomycin C-DNA bonding.
AUTHOR(S): Li, Ven-Shun; Reed, Matthew; Zheng, Yi; Kohn, Harold; Tang, Moon-shong (1)
CORPORATE SOURCE: (1) Department of Environmental Medicine, New York University, Tuxedo, NY, 10987 USA
SOURCE: Biochemistry, (March 14, 2000) Vol. 39, No. 10, pp. 2612-2618.
ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
TI C5 **cytosine methylation** at CpG sites enhances sequence selectivity of mitomycin C-DNA bonding.
AB. . . the degree of UvrABC cutting represents the extent of drug-DNA bonding. Using this method we determined the effect of C5 **cytosine methylation** on the DNA monoalkylation by MC and the related analogues N-methyl-7-methoxyaziridinomitosene (MS-NMA) and 10-decarbamoylmitomycin C (DC-MC). We have found that C5 **cytosine methylation** at CpG sites greatly enhances MC and MS-NMA DNA adduct formation at those sites while reducing adduct formation at non-CpG sequences. In contrast, although DC-MC DNA bonding at CpG sites is greatly enhanced by CpG **methylation**, its bonding at non-CpG sequences is not appreciably affected. These cumulative results suggest that C5 **cytosine methylation** at CpG sites enhances sequence selectivity of drug-DNA bonding. We propose that the **methylation** pattern and status (hypo- or hyper**methylation**) of genomic DNA may determine the cells' susceptibility to MC and its analogues, and. . .

L12 ANSWER 10 OF 18 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2001:109997 SCISEARCH
THE GENUINE ARTICLE: 393BM
TITLE: AFLP-based detection of DNA **methylation**
AUTHOR: Xu M L (Reprint); Li X Q; Korban S S
CORPORATE SOURCE: Univ Illinois, Dept Nat Resources & Environm Sci, Urbana, IL 61801 USA; Univ Illinois, Dept Nat Resources & Environm Sci, Urbana, IL 61801 USA
COUNTRY OF AUTHOR: USA
SOURCE: PLANT MOLECULAR BIOLOGY REPORTER, (DEC 2000) Vol. 18, No. 4, pp. 361-368.
Publisher: INT SOC PLANT MOLECULAR BIOLOGY, UNIV GEORGIA, DEPT BIOCHEMISTRY, ATHENS, GA 30602 USA.
ISSN: 0735-9640.

DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 17
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB By using the isoschizomers Hpa II and Msp I which display differential sensitivity to **cytosine methylation**, a modified **amplified** fragment length polymorphism (AFLP) technique has been developed to investigate DNA **methylation** profiles in eukaryotic organ isms. Genomic DNA was digested with a mixture of EcoR I and one of the isoschizomers, and ligated to oligonucleotide adapters. After two rounds of selective **PCR amplification**, followed by DNA separation on a Long Ranger gel **electrophoresis**, a subset of restriction fragments can be displayed on an X-ray film. Comparison of AFLP banding patterns between Hpa II and Msp I revealed the extent of DNA **methylation**. The technique has been successfully applied in this study to investigate DNA **methylation** profiles of apple (Malus domestica cv. Gala) genomic DNA extracted from leaves of field-grown adult trees and in vitro-grown shoot cultures. The results showed that up to 25 percent of AFLP bands were derived from **methylated** sequences, and among those, a few bands unique to either adult trees or in vitro shoots were observed. These results demonstrated that this protocol is effective in identifying **methylated** DNA profiles.

STP KeyWords Plus (R): **CYTOSINE METHYLATION**; RESISTANCE; GENE; **AMPLIFICATION**; SEQUENCES; PATTERNS; MARKERS; FUNGI

L12 ANSWER 11 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:339856 BIOSIS
DOCUMENT NUMBER: PREV200000339856
TITLE: **PCR amplification** in bisulfite methyl**cytosine** mapping in the GC-rich promoter region of amyloid precursor protein gene in autopsy human brain.
AUTHOR(S): Nagane, Yuriko; Utsugisawa, Kimiaki; Tohgi, Hideo (1)
CORPORATE SOURCE: (1) Department of Neurology, Iwate Medical University, 19-1 Uchimaru, Morioka, Iwate, 020-8505 Japan
SOURCE: Brain Research Protocols, (April, 2000) Vol. 5, No. 2, pp. 167-171. print.
ISSN: 1385-299X.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB. . . in DNA 5-methyldeoxycytidine pattern influence gene expression for certain mammalian genes in development, differentiation, carcinogenesis, and aging. Detection of DNA **methylation** at the promoter region, which generally represses transcription activity, is one important element in studying changes in molecular expression with aging and age-associated

disorders. Bisulfite genomic sequencing is a useful method for mapping **methylat**ed **cytosine**s. However, **PCR amplif**ication for bisulfite-treated DNA does not yield a sufficient amount of products that have a sufficient level of. . .

IT Methods & Equipment

ABI 373 STRETCH autosequencer: Perkin Elmer cetus, laboratory equipment; Horizon 11.14 gel **electrophoresis** apparatus: Gibco BRL, laboratory equipment; **PCR** [polymerase chain reaction]: DNA **amplif**ication, DNA **amplif**ication method, in-situ recombinant gene expression detection, sequencing. . . equipment; bisulfite genomic sequencing: Recombinant DNA Technology, gene sequencing method, sequencing techniques; bisulfite methyl**cytosine** mapping: gene mapping, gene mapping method; **electrophoresis**: electrophoretic techniques, separation method; ethidium bromide staining: staining method, staining/visualization

L12 ANSWER 13 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:200213 BIOSIS

DOCUMENT NUMBER: PREV200000200213

TITLE: Opposite effects of **methylat**ed and fluorinated **cytosine** on the reactivity of CpG with benzo(A)pyrene diolepoxide (BPDE) in oligonucleotides.

AUTHOR(S): Tomasz, Maria (1); Das, Arunangshu (1)

CORPORATE SOURCE: (1) Department of Chemistry, Hunter College, City University of New York, 695 Park Ave, New York, NY, 10021 USA

SOURCE: Abstracts of Papers American Chemical Society, (2000) Vol. 219, No. 1-2, pp. TOXI 73.
Meeting Info.: 219th Meeting of the American Chemical Society. San Francisco, California, USA March 26-30, 2000 American Chemical Society
. ISSN: 0065-7727.

DOCUMENT TYPE: Conference

LANGUAGE: English

SUMMARY LANGUAGE: English

TI Opposite effects of **methylat**ed and fluorinated **cytosine** on the reactivity of CpG with benzo(A)pyrene diolepoxide (BPDE) in oligonucleotides.

IT . . .

(Biochemistry and Molecular Biophysics); Toxicology

IT Diseases

cancer: neoplastic disease

IT Chemicals & Biochemicals

CpG; DNA; benzo[a]pyrene diolepoxide; fluorinated **cytosine**; **methylat**ed **cytosine**; oligonucleotides; p53

IT Alternate Indexing
Neoplasms (MeSH)
IT Methods & Equipment
HPLC [high performance liquid chromatography]: separation method
IT Miscellaneous Descriptors
gene mutation; Meeting Abstract

L12 ANSWER 18 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
ACCESSION NUMBER: 1985:406807 BIOSIS
DOCUMENT NUMBER: BA80:76799
TITLE: ANALYSIS OF PRODUCTS OF DNA MODIFICATION BY METHYLASES A
PROCEDURE FOR THE DETERMINATION OF 5 METHYL**CYTOSINE** AND N-4
METHYL**CYTOSINE** IN DNA.
AUTHOR(S): BUTKUS V V; KLIMASAUSKAS S J; JANULAITIS A A
CORPORATE SOURCE: INSTITUTE OF APPLIED ENZYMOLOGY, 232028 VILNIUS, FERMENTU
8, LITHUANIAN SSR, USSR.
SOURCE: ANAL BIOCHEM, (1985) 148 (1), 194-198.
CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Although many different methods are used for the identification of **methylat**ed heterocyclic bases in DNA not all of them possess the ability to discriminate N4-methyl**cytosine** (m4C) and 5-methyl**cytosine** (m5C). Therefore, some of the methods need reexamination. This paper reinvestigates some chromatographic systems TLC, paper chromatography and **electrophoresis**) most widely used in the analysis of minor bases occurring in nucleic acids according to their ability to separate m4C. . . sample and a chromatographic system for its analysis was developed. The recommended chromatographic systems may be used for the simultaneous separation of not only m4C and m5C, but also both **methylat**ed **cytosine**s together with N6-methyladenine and 7-methylguanine.

L18 ANSWER 5 OF 19 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-02844 BIOTECHDS
TITLE: Analyzing **methylation** of **cytosine** bases in genomic DNA, by chemically treating DNA to convert **cytosine** to uracil, **amplif**ying DNA and hybridizing with fluorophore labeled probes, and detecting fluorescence

polarization;
DNA primer, DNA probe, DNA chip and bioinformatics for high throughput screening and disease diagnosis

AUTHOR: BERLIN K

PATENT ASSIGNEE: EPIGENOMICS AG

PATENT INFO: WO 2002061123 8 Aug 2002

APPLICATION INFO: WO 2002-EP922 29 Jan 2002

PRIORITY INFO: DE 2001-1004937 29 Jan 2001; DE 2001-1004937 29 Jan 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-674824 [72]

TI Analyzing **methylation** of **cytosine** bases in genomic DNA, by chemically treating DNA to convert **cytosine** to uracil, **amplif**ying DNA and hybridizing with fluorophore labeled probes, and detecting fluorescence polarization;
DNA primer, DNA probe, DNA chip. . .

AB DERWENT ABSTRACT:

NOVELTY - Analyzing **methylation** of specific **cytosine** bases in genomic DNA samples, comprising chemically treating DNA to convert **cytosine** to uracil, **amplif**ying treated DNA using oligonucleotide primer, contacting **amplif**ied sequence (AS) with fluorophore labeled oligonucleotide probes, hybridizing flurophore labeled probes to AS, and detecting fluorescence polarization of labeled probes, is new.

DETAILED DESCRIPTION - Analysis (M) of **methylation** of specific **cytosine** bases in genomic DNA samples, comprising: (a) chemically treating the genomic DNA for converting **cytosine** to uracil or a similar acting base regarding the base pairing behavior in the DNA duplex, 5 methyl**cytosine** remains unmodified; (b) the chemically treated DNA is **amplif**ied using at least one oligonucleotide (type A) as primer in a. . . one or more pairs of oligonucleotides (type B), which hybridize to the positions which are to be examined regarding their **methylation** status in the genomic DNA sample in which one oligonucleotide of each pair hybridizes preferentially in each case if in the genomic DNA sample the position was **methylat**ed, while the other oligonucleotide of the pair hybridizes preferentially, if the position was un**methylat**ed, each oligonucleotide of a pair is. . . used one determines the degree of polarization. An INDEPENDENT CLAIM is also included for a diagnostic kit for detecting the **methylation** of specific **cytosine** bases in genomic DNA samples comprising one or more pairs of fluorescent labeled oligonucleotides designed to hybridize to a target. . . oligonucleotide hybridization is detected by an increase in fluorescence polarization. The fluorophore is a 5'-carboxyfluorescein, 6-carboxy-X-rhodamine, N,N,N',N'-tetramethyl-6-carboxy-X-rhodamine, BODIPY, Texas Red, **Cy3**, **Cy5**, FAM, fluorescein isothiocyanate (FITC), DAPI, HEX or TET. The DNA sample

is cleaved prior to bisulfite treatment with restriction endonucleases.. . . steel, iron, copper, nickel, silver or gold, prior to hybridization with the Type B oligonucleotide. The information generated about the **methylation** status at the target site is provided to a computing device comprising one or more databases or learning algorithms.

USE - (M) is useful in analysis of **methylation** of specific **cytosine** bases in genomic DNA samples isolated from mammalian sources e.g. cell lines, blood, sputum, feces, urine, cerebrospinal fluid, tissue embedded. . . density DNA chip analysis, provides a cost-effective method of analysis and the results are obtainable minutes after carrying out the **methylation** specific reaction. (30 pages)

L18 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:633164 CAPLUS
DOCUMENT NUMBER: 139:160777
TITLE: Identification of **methylat**ed **cytosine**s in DNA using differential reactivity with bisulfite and using probes for parallel analysis of many potential sites
INVENTOR(S): Garner, Harold R.; Minna, John D.; Luebke, Kevin J.; Balog, Robert P.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 210 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003152950	A1	20030814	US 2002-184085	20020627

PRIORITY APPLN. INFO.: US 2001-301370P P 20010627

TI Identification of **methylat**ed **cytosine**s in DNA using differential reactivity with bisulfite and using probes for parallel analysis of many potential sites

AB The present invention provides a high-throughput method for the parallel anal. of many potential sites of chem. modification (e.g., **methylation**) in DNA. It makes use of chem. treatment of the DNA to alter its sequence in a way that depends upon the modification of interest and subsequent anal. of the resulting sequence by hybridization to an array of probes. Treatment with sodium bisulfite converts **methylat**ed and un**methylat**ed DNA to different sequences. Un**methylat**ed **cytosine**s in DNA react with sodium bisulfite to yield deoxyuridine, which behaves as thymidine in hybridization and

enzymic template-directed polymn., whereas **methylated** **cytosines** are unreactive and behave as **cytosine** in hybridization and polymn. A device, comprising the array of probes, is provided by the invention, and principles and methods for its design and fabrication are also provided. A procedure for probe design is provided in which a parent probe created from the complementary sequence is filtered to remove probes that are deemed not to be suitable for re-sequencing anal., and each parent probe is used as a template to create new probes to query for possible changes at a particular position in the ref. sequence. Thus, **methylation** is analyzed in a region of the promoter for the tumor suppressor gene p16. A 1280-member 20-mer probe set for a 145-bp region of the promoter of p16 is provided.

ST DNA **methylation** analysis bisulfite reactivity probe set; gene p16 **methylation** analysis bisulfite probe set